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NanoPULSE: An Innovative Microfluidics-Based Active Mixer for Lipid Nanoparticle Formulation

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ABSTRACT: The field of nanomedicine has been attracting experts from various disciplines, aiming to develop nanocarriers capable of encapsulating potent drugs and nucleic acids to treat or prevent critical diseases, exemplified by RNA-LNP therapies. This progress has created a demand for tools that enables the precise synthesis of **monodisperse lipid nanoparticles (LNPs)**. However, **the field currently lacks a reliable method to scale production** from laboratory research (μL scale) to large-scale clinical production (10+L), hindering the translation from drug development to clinical application. Here, we introduce Nanopulse, a patented **microfluidic-based active micromixer prototype (NanoPULSE) leveraging Taylor-Aris Dispersion**, designed to meet this urgent manufacturing need in the pharmaceutical industry.

PROBLEMATIC :

- Small volume limitation:** large scale methods (IJM, T-mixer...) cannot be used at lab scale (< 1 mL).
- Quality issues:** microfluidic techniques fail after a limited production time due to agglomeration issues, leading to higher PDIs and rendering scale-up difficult.
- Scalability bottleneck:** traditional micromixing techniques rely on chaotic mixing, resulting in flow-rate-depending RNA-LNP characteristics.

1 Solution 1: Ensuring Low volume production

NanoPULSE is inspired by microfluidic systems and allows for highly controlled LNP synthesis at low volumes (<1 mL) for screening & preclinical development. This **highly scalable micromixer** is based on the **Taylor-Aris Dispersion** of sequentially injected fringes of fluids.

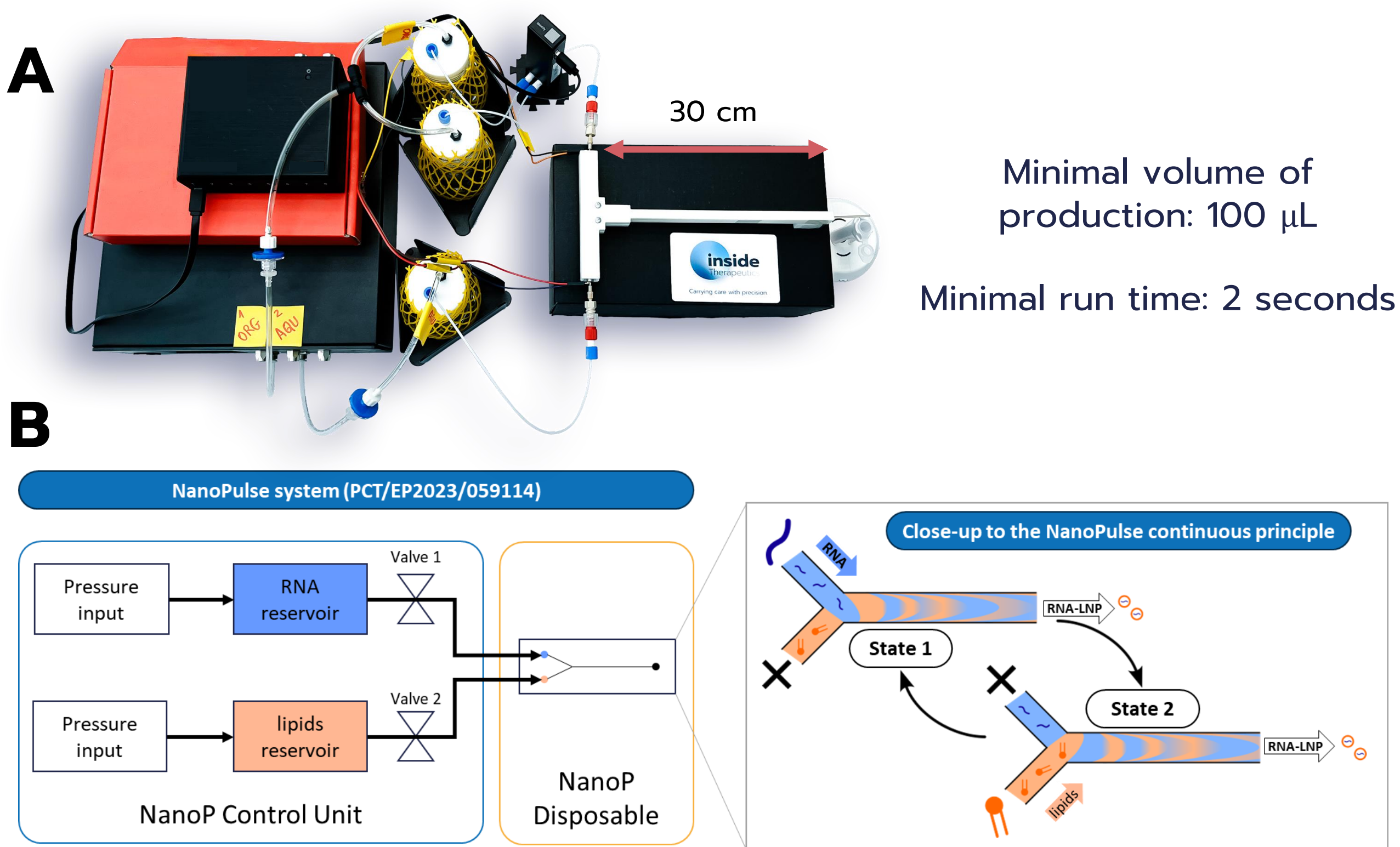


Figure 1. A) NanoPULSE prototype system and **B)** schematic of the working principle with footprints and minimal production volumes.

2 Solution 2: Maintaining constant COAs during scale up

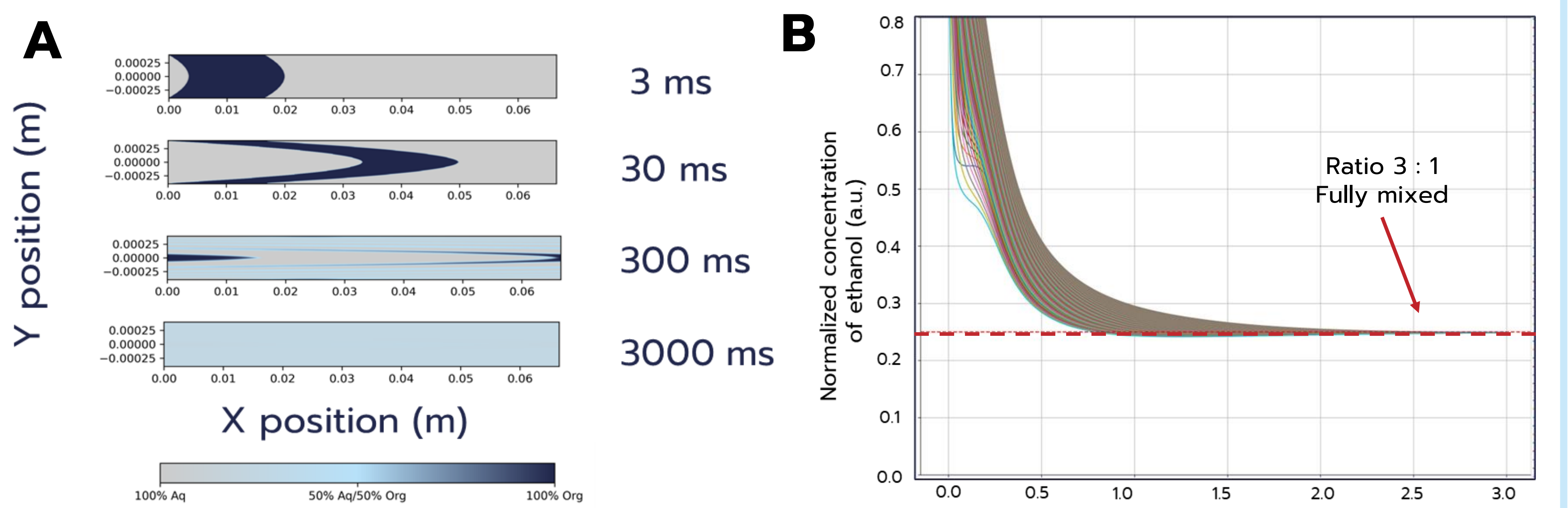


Figure 2. A) Lagrangian simulations at TFR of 1 L/h and FRR = 3. **B)** Resulting evolution of ethanol concentration over time, averaged on all control volumes. **C)** Experimental data obtained after producing PC : Cholesterol (2 : 1) liposomes at 6 mg/mL. Size and PDI were measured by DLS.

HIGHLIGHTS

- NanoPULSE** is a unique active mixing technique allowing **continuous production** of RNA-LNP at both **low and high flow rates and volumes**.
- Experimental data show **constant size** over a 6-fold increase in flow rate and **high monodispersity** (PDI < 0.2).
- NanoPULSE has been proven to be **highly repeatable** and **100% agglomeration free** in the mixing channel even after long production times.

3 Solution 3: Allowing for Continuous production at large scale

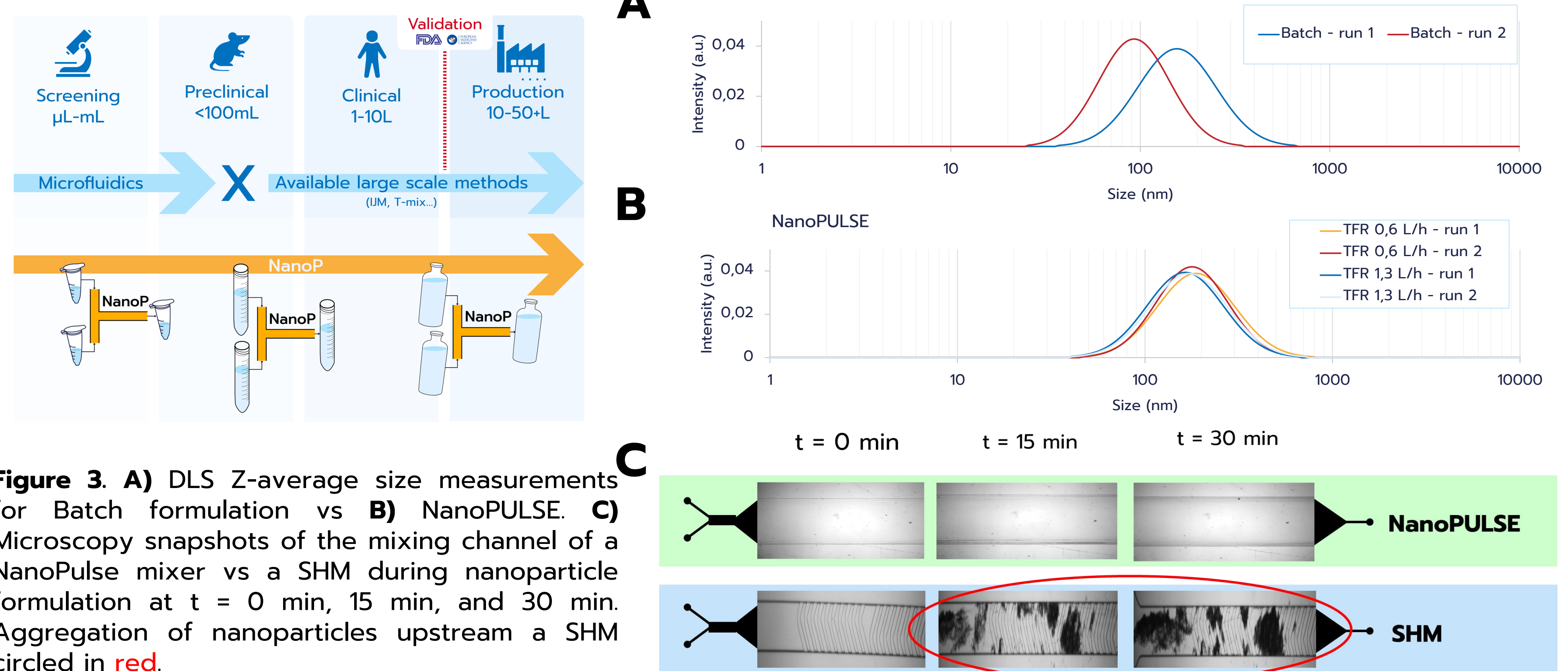


Figure 3. A) DLS Z-average size measurements for Batch formulation vs **B)** NanoPULSE. **C)** Microscopy snapshots of the mixing channel of a NanoPulse mixer vs a SHM during nanoparticle formulation at t = 0 min, 15 min, and 30 min. Aggregation of nanoparticles upstream a SHM circled in red.

CONCLUSIONS & PERSPECTIVES: NanoPULSE introduces a novel **active mixing technology** that enables **flow-rate-independent synthesis** through **high-frequency valve switching** using **Taylor-Aris dispersion**. This allows for **precise control** over small sample production **at the μL scale** and seamless transition to **continuous large-scale production**. The active mixing properties ensure the synthesis of nearly identical LNPs across various scales and flow rates. Future validation steps include increasing flow rates with higher valves frequencies, correlating Lagrangian simulation results with experimental data, and confirming the consistency of LNPs' critical attributes such as **zeta potential and encapsulation efficiency**. We also aim to validate the continuous, **agglomeration-free production** of liposomes at large scale. This would confirm NanoPULSE's capability to support all drug development stages, from sub-mL to large-scale continuous production, while ensuring scalability.

